

A rare *cD-/(C)D-* phenotype in an individual and production of anti-Rh17-like

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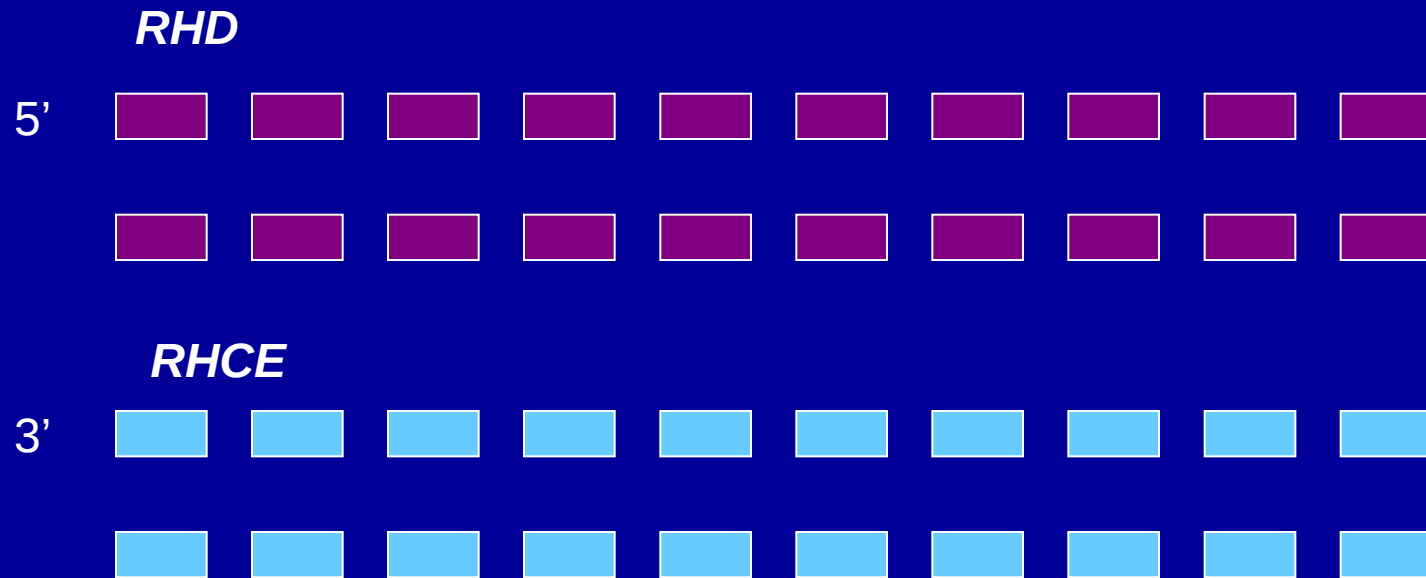
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2: ARCBS, Brisbane, Australia.

3: HKRCBTS, Kowloon, Hong Kong

Introduction

- Rh antigens are encoded by *RHD* and *RHCE*
- These 10 exon genes are closely linked on the short arm of chromosome 1 and share approximately 94% sequence homology



Hybrid Haplotypes

- Exchange of genetic material between *RHD* and *RHCE* can result in hybrid genes
- This can cause weakened expression of some common Rh antigens and some rarer low frequency Rh antigens may also be expressed e.g the R^N haplotype
- Lack of expression of RhCc or RhEe with normal or enhanced expression of RhD can also occur e.g –D–/–D–

RHCE-D-CE Hybrid Haplotypes

RHD



RHCE



Representation of R^N haplotype where exon 4 of *RHCE* has been replaced by exon 4 of *RHD*

RHD



RHCE



Representation of –D– haplotype where exons 2-9 of *RHCE* were replaced with the corresponding exons of *RHD*.

Case Study

- A 37 year old female patient of chinese descent, was referred to IBGRL from Hong Kong via Brisbane, Australia.
- Rh Phenotype D+, C+^{wk}, c+, E–, e–
- In addition patients' cells Rh:–46 but their “anti-Rh32 had not worked”
- Plasma compatible with Rh_{null} and –D–/–D– cells
- Two examples of Rh:32, –46 cells were found to react weakly

Rh Phenotyping

Extended Rh Phenotype	Anti-D	Anit-C	Anti-c	Anti-E	Anti-e	Anti-Rh 46	Anti-Rh 32	Anti-hr	Anti-hr ^B	Anti-Rh51-like	Anti-Rh 17	Anti-Rh29
Patient	+	(+)	* +	-	-	-	-	-	-	-	-	+
Pos Ctl	+	+	+	+	+	+	+	+	+	+	+	+
Neg Ctl	-	-	-	-	-	-	-	-	-	-	-	-

*Variable reactivity with anti-c
 () detected by absorbtion elution only

Genetics

- Sequence specific PCR results for the patient indicated D+ C– c+
- Allelic discrimination genotyping gave no result for E and e
- Gene specific PCR was attempted for all 10 exons of *RHD* and *RHCE*
- No PCR product was obtained for exons 4-9 of *RHCE*
- Genomic DNA sequencing showed:
 - No mutations in *RHD*
 - No mutations in exons 1,2,3 and 10 of *RHCE*

Family Serology

Rh Phenotype	Anti-D	Anit-C	Anti-c	Anti-E	Anti-e
Mother	+	-	±*	-	+
Father	+	+	-	-	+
Brother	+	+	-	-	+
Sister	+	- †	±*	-	-
Pos Ctl	+	+	+	+	+
Neg Ctl	-	-	-	-	-

*Variable reactivity with anti-c

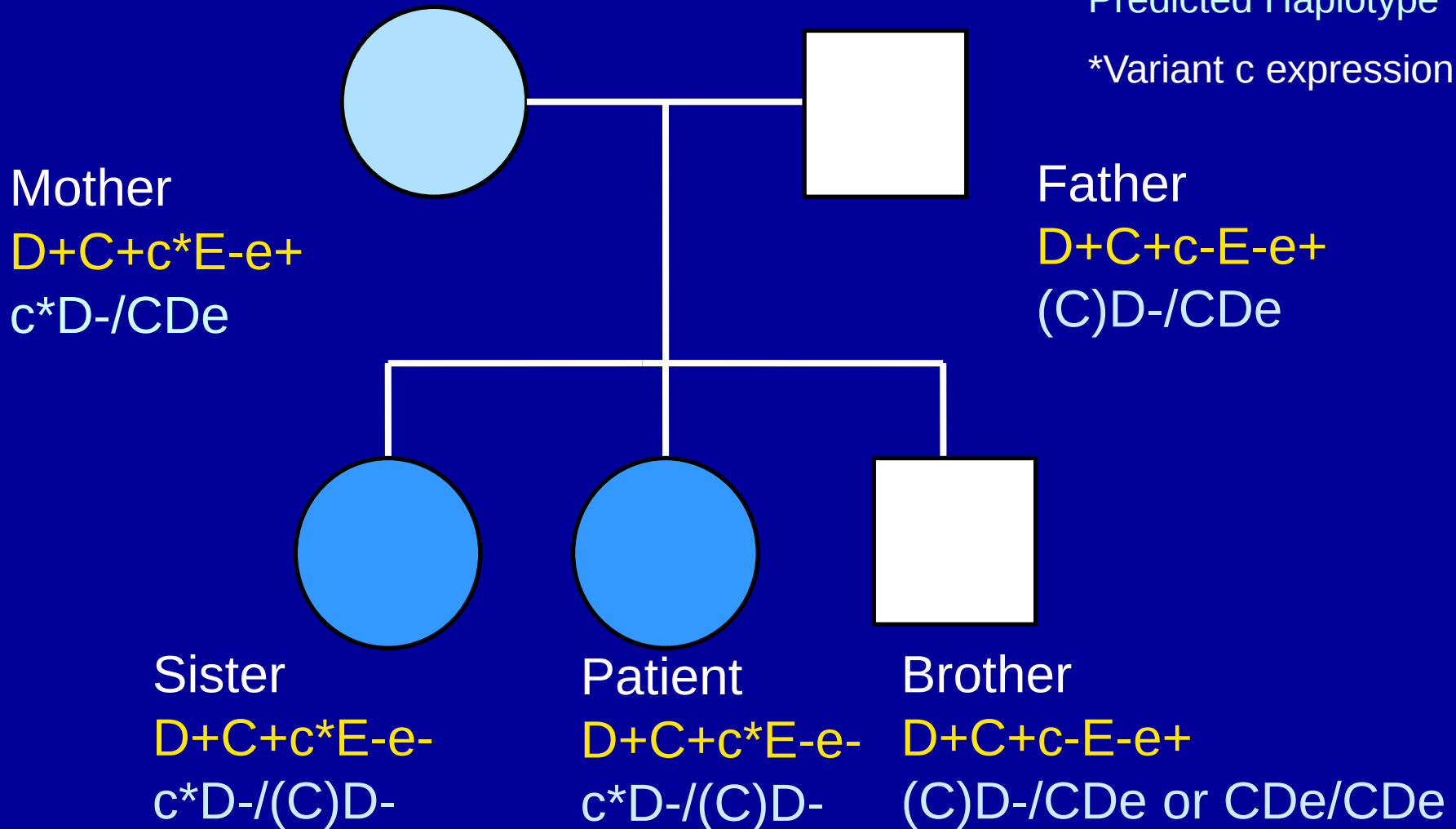
† No absorbion elution studies performed

Family genetics

Sequencing results

Predicted Haplotype

*Variant c expression



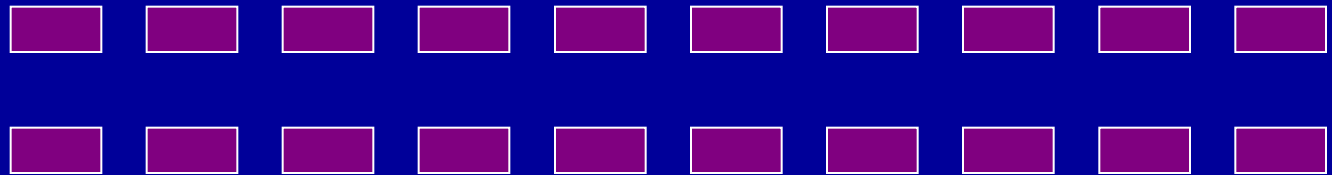
Results

- Sequencing results suggest the presence of the hybrid allele *RHCE*CE-D(4-9)-CE*
- Presence of some RhCcEe antigens indicates a gene that enables expression of Rh protein
- Heterozygosity for *RHCE*C* and *RHCE*c* indicates the presence of 2 *RHCE* alleles both lacking exons 4-9

Why did allelic discrimination PCR give “no result” for E and e

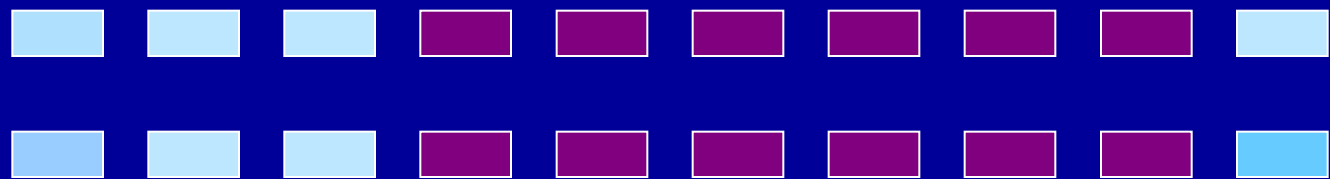
RHD

5'



RHCE

5'

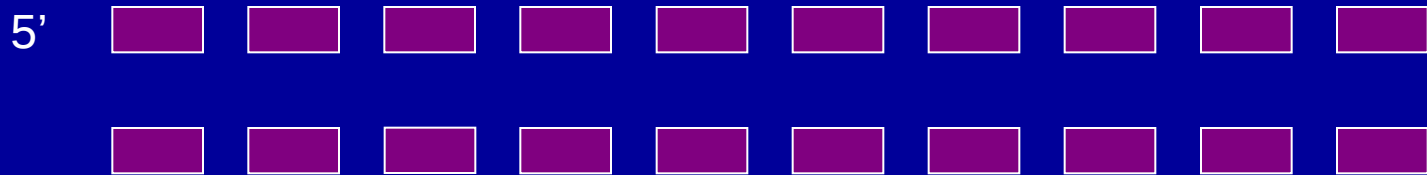


RhE

Rhe

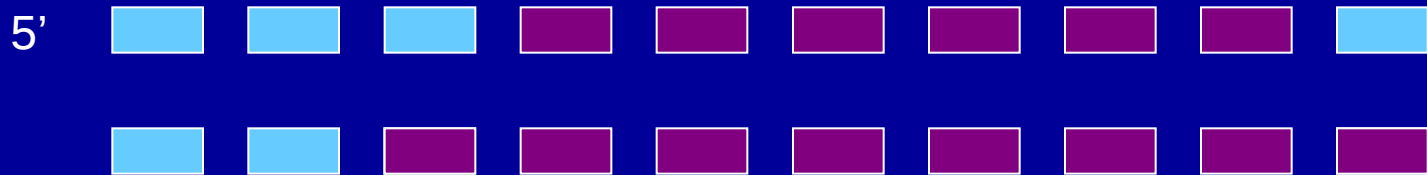
Why was the sequence specific PCR C—?

RHD



RHCE

Rhc



RhC

↑
109bp insert in intron 2

Interpretation

- Expression of E and e is dependant on the Pro226Ala polymorphism present in exon 5 of *RHCE*
- c expression is determined by Pro103 in exon 2 of *RHCE*.
- C expression requires not only Ser103 but also RHCcEe amino acids from exon 5

Interpretation

- 3 examples anti-Rh17 (total RhCcEe) were negative with the patient's cells despite the presence of some RhCcEe antigen expression.
- This could suggest that there is not enough RhCcEe protein present or that is so altered that this broad specificity antibody cannot bind

Conclusions

- The patients' and her sister's unusual phenotype are most likely the result of compound heterozygosity for 2 hybrid *RHCE-D-CE* genes both lacking at least exons 4-9
- The antibody that this patient has produced is best described as anti-Rh17-like

Further Investigation

- Genomic DNA sequencing cannot distinguish between homozygous and hemizygous
- cDNA sequencing could reveal the precise gene organisation in these individuals

THANK-YOU
Any Questions

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